Mesenchymal stem cell transplantation for diffuse alveolar hemorrhage in SLE

Jun Liang, Fei Gu, Hong Wang, Bingzhu Hua, Yayi Hou, Songtao Shi, Liwei Lu and Lingyun Sun

Background. A 19-year-old girl was diagnosed with systemic lupus erythematosus, arthritis, malar rash, positive antinuclear antibody test and high levels of antibodies to double-stranded DNA. Two months after diagnosis, the patient presented with a sudden drop in blood hemoglobin level. Several days later, she developed bloody sputum, rapidly progressive dyspnea and hypoxemia. High-resolution CT showed diffuse alveolar infiltrates in both lung fields.

Investigations. Physical examination, complete blood count, erythrocyte sedimentation rate, urinalysis, 24-h urine protein excretion, fecal occult blood test, D-dimer test, acid hemolysis test, activated partial thromboplastin time and prothrombin time, direct and indirect Coombs tests, bone marrow smear, arterial blood gas, sputum smear and culture, and high-resolution CT scan of the chest.

Diagnosis. Diffuse alveolar hemorrhage associated with systemic lupus erythematosus.

Management. The patient did not respond to pulsed methylprednisolone therapy (two course of 500 mg per day for 3 days) and immunoglobulins (20 g per day for 5 days). The patient was referred to a specialist treatment center for allogenic transplantation using umbilical cord-derived mesenchymal stem cells. She underwent transplantation with an infusion of 8×10^7 mesenchymal stem cells. After showing dramatic improvements in her clinic conditions, oxygenation level, radiographic and hematological status, the patient was discharged from hospital approximately 1 month after undergoing transplantation.

J. Liang *et al. Nat. Rev. Rheumatol.* advance online publication XX XXX 2010; doi:10.1038/nrrheum.2010.80

The case

A previously healthy 19-year-old girl presented to a local hospital with a 2-week history of arthritis, headache and hair loss. Physical examination showed a normal temperature and normal oxygen saturation level of 98% when breathing room air, malar rash and mouth ulcers. Lung, heart and abdominal examinations were normal. Laboratory analysis revealed a normal white blood cell count and hemoglobin level (11.5 g/dl; normal range 11–15 g/dl), a low platelet count (50×10^9/l; normal range $100–300 \times 10^9$/l), normal serum creatinine and serum albumin levels, a high erythrocyte sedimentation rate (ESR; 56 mm/h, normal range 0–20 mm/h), and low levels of complement C3 (0.32 g/l; normal range 0.8–1.6 g/l) and complement C4 (0.02 g/l, normal range 0.2–0.4 g/l). Urinalysis showed hematuria and proteinuria. The mean 24-h urine protein excretion was 349.3 mg (standard reference value <300 mg). In addition, the patient's antinuclear antibody (ANA) was positive with high titer of 1:1,200 (homogeneous pattern), and the level of antibodies against double-stranded (ds) DNA was 564.6 IU/ml (standard reference value <50 IU/ml). On the basis of these findings a diagnosis of systemic lupus erythematosus (SLE) was made according to the American College of Rheumatology criteria, and therapy was initiated comprising intravenous methylprednisolone 40 mg per day and oral hydroxychloroquine 400 mg per day.

Approximately 6 weeks later, the patient was free of joint pain and headache, but presented with pale mucosa and shortness of breath. A complete blood count showed a sudden drop in hemoglobin level from 11.5 g/dl to 5.7 g/dl, with a moderate increase in platelet count to 56×10^9/l. Fecal occult blood, D-dimer and acid hemolysis tests were negative. Activated partial thromboplastin time and prothrombin time were within normal limits, and direct and indirect Coombs tests were negative. A bone marrow smear showed marked hypercellularity. No evidence of active bleeding from the gastrointestinal, genitourinary or nasal areas was found. Two weeks later, methylprednisolone 160 mg per day was added to the treatment regimen for 4 days. On cessation of methylprednisolone, the patient's platelet count increased to 66×10^9/l and the ESR was markedly reduced to 4 mm/h, but the hemoglobin level dropped further to 3.9 g/dl. The patient had a sudden onset of cough and bloody sputum. Auscultation revealed fine inspiratory crackles in both lung bases. A high-resolution CT (HRCT) scan of the chest revealed bilateral, diffuse, alveolar infiltrates (Figure 1a). A sputum smear and culture were both negative for bacteria, fungi and acid-fast bacilli. Blood and urine cultures were also

Competing interests
The authors declare no competing interests

Department of Rheumatology and Immunology, The Affiliated Drum Tower Hospital of Nanjing University Medical School, 321 Zhongshan Road, Nanjing, Jiangsu 210008, China (**J. Liang**, **F. Gu**, **H. Wang**, **B. Hua**, **L. Sun**). Immunology Laboratory, Nanjing University Medical School, 22 Hankou Road, Nanjing, Jiangsu 210093 (**Y. Hou**), China. Center for Craniofacial Molecular Biology, University of Southern California School of Dentistry, 2250 Alcazar Street, CSA 103, Los Angeles, CA 90033, USA (**S. Shi**). Department of Pathology and Center of Infection and Immunology, The University of Hong Kong, Pokfulam Road, Hong Kong, China (**L. Lu**).

Correspondence to:
L. Sun
lingyunsun2001@yahoo.com.cn

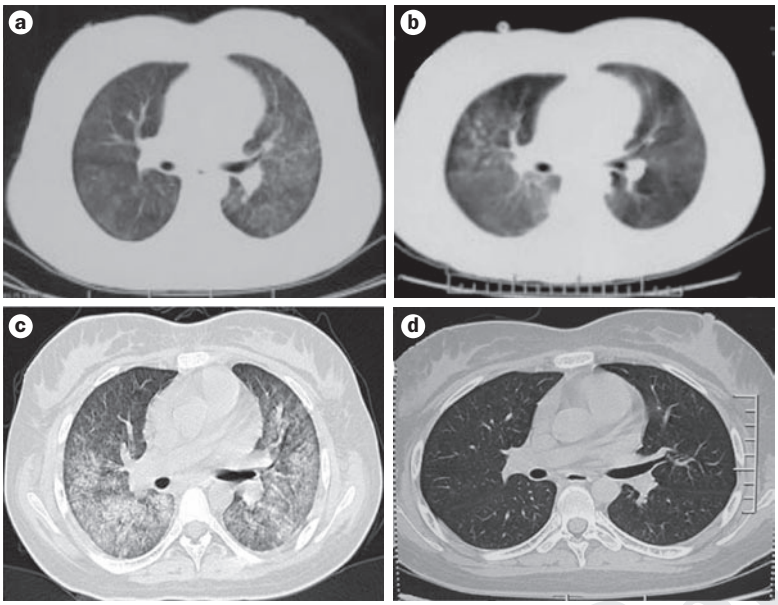

Figure 1 | Chest HRCT scans before and after UC-MSCT. **a** | An initial HRCT scan of the chest shows bilateral, diffuse, alveolar infiltrates. **b** | Two weeks later, after the first course of treatment with pulsed methylprednisolone, a repeat HRCT scan of the chest shows progressive lung infiltrates. **c** | The second course of pulsed methylprednisolone 4 days later resulted in no improvements. **d** | Nine days after UC-MSCT, an HRCT scan of chest using a 3 mm collimation of the lung shows complete resolution of the lung infiltrates. Abbreviations: HRCT, high-resolution CT; UC-MSCT, umbilical cord mesenchymal stem cell transplantation.

negative for bacterial growth. A diagnosis of diffuse alveolar hemorrhage (DAH) associated with SLE was made, and the patient was administered pulsed intravenous methylprednisolone 500 mg per day for 3 days and intravenous immunoglobulin 20 g per day for 5 days, in addition to prophylactic antibiotics and antifungal agents. Multiple transfusions of washed red blood cells were also performed. Despite these therapeutic measures, however, the patient's pulmonary condition did not improve.

Nine weeks after first being admitted, a repeat HRCT of the chest showed progressive lung infiltrates (Figure 1b). In addition, dyspnea and voluminous hemoptysis were observed. Measurement of arterial blood gas showed severe hypoxemia with a partial pressure of oxygen of 39 mmHg and an oxygen saturation level of 78% despite administration of 100% oxygen. The hemoglobin level increased slightly to 5.1 g/dl and the platelet count was normal (114×10^9/l). The patient then received noninvasive mechanical respiratory support and 500 mg per day pulsed intravenous methylprednisolone treatment for a further 3 days. However, there were no improvements in hypoxemia or dyspnea, and an HRCT scan showed no improvements (Figure 1c). The patient was transferred to a specialist treatment center for umbilical cord-derived mesenchymal stem cell transplantation (UC-MSCT) 4 days later.

At the time of admission to the specialist center, the patient looked pale and was tachypneic. Physical examination showed an elevated pulse rate of 98 beats per min, a raised respiratory rate of 30 breaths per min, and a low oxygen saturation level of 71% (normalized by a facial mask and noninvasive mechanical ventilation). Diffuse crackles and tachycardia were found on auscultation, and lower extremity edema was also apparent. Laboratory tests revealed a low hemoglobin level of 56 g/dL, a high reticulocyte count of 9.98% (normal range 0.5–1.5%), and low levels of C3 complement (0.44 g/l) and C4 complement (0.11 g/l). The ESR, C-reactive protein (CRP) level and platelet counts were at normal levels. Tests for antineutrophil cytoplasmic antibodies, ANA and anti-dsDNA antibodies were all negative. Urinalysis was normal and the 24-h urinary protein excretion was 376 mg.

For UC-MSCT, 8×10^7 cells (2×10^6 cells per kg of the patient's weight) were infused, and intravenous methylprednisolone 40 mg per day was administered concomitantly. Following treatment, the patient showed dramatic improvements in respiratory failure, oxygenation level, and radiographic and hematological status. One day following UC-MSCT, the patient's oxygen saturation level increased to 91% and arterial blood gas measures showed moderate improvements in hypoxia with a partial pressure of oxygen of 75 mmHg. Over the following days, progressive improvements were found in all of these parameters, and mechanical respiratory support was removed 5 days after UC-MSCT. An oxygen supplement (3l/min) was administered by nasal catheter for a further 8 days. A follow-up HRCT scan of the chest 4 days later (9 days after UC-MSCT) showed complete resolution of the lung infiltrates (Figure 1d). The oxygen supplement was stopped 3 days after the HRCT scan and oxygen saturation level stabilized at 96–98%. Hemoglobin levels fluctuated between 8.5 g/dl and 9.0 g/dl. A further 2 weeks later, laboratory data revealed a stable hemoglobin level of 8.9 g/dl, a complement C3 level of 0.79 g/l and a complement C4 level of 0.16 g/l. The patient was discharged from hospital 1 week later (approximately 5 weeks after UC-MSCT **[Au:OK?]**) in a stable condition with a regimen of prednisolone 30 mg per day.

Approximately 6 weeks after being discharged, the patient returned for a scheduled follow-up visit. At this point, hemoglobin levels remained stable at 8.9 g/dl and the HRCT scan showed no obvious abnormalities. The patient was prescribed oral prednisone 20 mg per day, oral ciclosporin 150 mg per day and intravenous cyclophosphamide 400 mg every other week.

Three months after being discharged, and despite combination therapy with prednisone, ciclosporin and cyclophosphamide, the patient was readmitted for dyspnea and hemoptysis. An HRCT scan again revealed diffuse bilateral pulmonary infiltrates. The patient received a second course of UC-MSCT, with the same doses of prednisolone and immunosuppressive agents. The treatment was well tolerated and the patient recovered. She was discharged with gradual tapering of dosages of prednisolone and immunosuppressive agents. No further relapse of DAH had occurred at 5-month follow-up, and the patient continued to receive treatment with oral prednisolone 10 mg per day, oral ciclosporin 75 mg per day and intravenous cyclophosphamide 400 mg every other week.

Diagnosis

DAH is an unusual but serious and often fatal complication of SLE, with an early mortality of at least 50%.[1] Female

patients with an average age of 29 years are most commonly affected.[2] The clinical presentation of DAH includes dyspnea, hemoptysis, hypoxemia and a sudden drop in hemoglobin level. HRCT often shows diffuse bilateral alveolar opacities. As described in this case, a diagnosis of DAH is usually made based on the presence of all these features and by the exclusion of other conditions such as antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis, Goodpasture syndrome (also known as anti-glomerular basement membrane disease), thrombotic thrombocytopenic purpura and infection.

The etiopathogenesis of SLE-associated DAH is not fully understood, although contributory factors are thought to include immune complex-mediated injury, thrombocytopenia, vasculitis, congestive heart failure, renal failure and infection.[3,4] The incidence of SLE-associated DAH is reported to range from 1.9% to 5.7%.[5,6] DAH can occur at any stage during the course of SLE, and frequently presents with concurrent fever, arthritis, myositis, and peripheral neuropathy.[6] Although scores on the SLE Disease Activity Index (SLEDAI) are raised in the presence of DAH, this has not been associated with increased mortality.[7] The most common extrapulmonary manifestation of SLE-associated DAH is renal involvement, including clinical nephritis, nephritic syndrome or acute renal failure.[7,8] In the present case, DAH developed when the symptoms of joint pain and headache treatment improved, and the signs and symptoms of DAH were the only manifestations of SLE. SLE disease activity, thrombocytopenia and renal failure were ruled out as possible major contributory factors for the development of DAH. We hypothesize that the patient had pulmonary capillaritis at the onset of SLE. The lesions present in the lung did not respond to treatment with high-dose intravenous methylprednisolone and, therefore, eventually developed into DAH.

Treatment

The therapeutic protocol for SLE-associated DAH is not well defined. Early treatment with high-dose intravenous corticosteroids is the mainstay of therapy, but this is not effective for every patient,[9] including the patient described in this report. When the patient was referred for UC-MSCT, her condition was so critical that treatment with cyclophosphamide, which often exerts a slow and moderate level of immunosuppression, was inadequate. After offering guidance and counseling to both the patient and her family, an agreement was reached to initiate an infusion of mesenchymal stem cells (MSCs).

MSCs are multipotential nonhematopoietic **[Au:OK?]** progenitor cells capable of differentiating into multiple lineages of cells, including osteoblasts, chondrocytes, myoblasts, adipocytes and endothelial cells. These cells are able to escape alloantigen recognition because of their low immunogenicity. However, they can also inhibit immune responses both *in vitro* and *in vivo*. This inhibiting effect of MSCs is related to the functional modulation of dendritic cells,[10] T cells[11,12] and natural killer cells.[12,13] The immunomodulatory properties of MSCs provide a rational basis for their application in the treatment of immune-mediated diseases such as graft-versus-host disease and autoimmune diseases.[14] In a previous study, our group demonstrated the functional abnormality of bone-marrow-derived MSCs from patients with SLE and lupus-prone MRL/lpr mice.[15] We hypothesized that SLE is, therefore, an MSC-mediated disease, which prompted us to further investigate the therapeutic effect of MSC transplantation (MSCT) in SLE.

In a mouse model of lupus, we found that MSCT was effective for inhibiting autoimmune disease,[16] resulting in marked reductions of proteinuria and serum levels of creatinine and antibodies against ds-DNA, and increased levels of complement C3. Histopathological examination showed marked decreases in the degree of glomerular sclerosis and interstitial fibrosis in MSCT-treated mice compared with nontreated controls. Immunohistochemical studies further revealed lowered expression of complement C3 in renal tissue after transplantation compared with lupus-prone mice treated with cyclophosphamide and with untreated mice. Following the observed clinical benefits of MSCT in a mouse model of lupus, we tested the efficacy of MSCT in four patients with SLE refractory to cyclophosphamide therapy.[17] All patients showed decreased SLEDAI scores and 24-h proteinuria, as well as improvements in serum levels of complement C3 compared with baseline. In addition, the percentage of regulatory T (T_{REG}) cells increased followed MSCT. These short-term clinical data suggest that allogenic MSCT could be a safe and feasible salvage therapy in these patients.[17]

In 2008, Zhao *et al.*[18] suggested that MSC engraftment can attenuate lung injury in a bleomycin-induced mouse model, and that this effect was dependent on the ability of the cells to engraft in the lung and to differentiate into alveolar epithelial cells. In addition, Gupta *et al.*[19] reported that treatment with MSCs in mice with experimental endotoxin-mediated acute lung injury provided a significant survival advantage compared with no treatment, possibly by reducing lung vascular permeability and the extent of pulmonary edema. The results of these studies encouraged our group to investigate whether administration of MSCs would have beneficial effects in patients with SLE and associated lung injury. UC-MSCs were chosen because these cells share most of the characteristics of bone marrow-derived MSCs but with the

Box 1 | Preparation of UC-MSCs

UC-MSCs were obtained from Jiangsu Stem Cell Bank, Jiangsu, China, and prepared for transplantation as follows: UCs were obtained from local maternity hospitals after normal deliveries. After having been minced into 1–2 mm^3 fragments, UCs were incubated with 0.075% collagenase type II for 30 min and then 0.125% trypsin for a further 30 min with gentle agitation at 37 °C. The obtained cells were plated at a density of $1\times10^6/cm^2$ in noncoated T-25 or T-75 cell culture flasks. The UC growth medium consisted of Dulbecco's modified Eagle's medium with low glucose and 5% fetal bovine serum. After 3 days of culture, nonadherent cells were removed and the medium was changed twice-weekly thereafter. Once 60–80% confluence had been reached, adherent cells were replated at a density of $1–10^4/cm^2$ in UC growth medium for expansion. After two passages, cells were harvested that expressed **[Au:OK?]** CD106, CD105, CD44 and CD29, but not CD34 or CD45. The capacity of MSCs to differentiate along adipogenic and osteogenic lineages was also assayed.

Abbreviations: MSC, mesenchymal stem cell; UC, umbilical cord.

distinct advantages of higher proliferation, improved accessibility and lower risk of viral contamination.[20,21] Box 1 provides further details on the preparation of UC-MSCs for infusion. In the present case, the patient showed considerable improvement 9 days after treatment with UC-MSCT. Considering that the patient had not responded to two cycles of high-dose steroids and showed rapid improvement after MSCT, we suggest that it was the MSCs, rather than the corticosteroids, that exerted a therapeutic effect.

The specific mechanisms by which MSCT might improve SLE-associated DAH are not known, and could be multifactorial. MSCs could, for example, act in an immunomodulatory capacity by producing soluble factors induced upon stimulation. An imbalance of cytokine homeostasis is a prominent feature of both experimental lupus and human SLE. Experimental data have shown that MSCs polarize the balance of type 1 and type 2 helper T (T_H1 and T_H2) cells via the secretion of cytokines such as interferon-γ and interleukin-4.[16] Another mechanism of action of MSCs could be related to an effect on the immune balance between T_{REG} cells and T_H17 cells. T_{REG} cells play a pivotal role in the maintenance of dominant self-tolerance, and the frequency and function of T_{REG} cells has been shown to be considerably deficient in patients with active SLE.[22] By contrast, T_H17 cells, which are inflammatory interleukin-17-producing cells, have been shown to augment inflammation and autoantibody production in the context of SLE.[23] We found that MSCT can recover T_{REG} cells and downregulate T_H17 cells.[17] A third mechanism could be direct cell replacement by the differentiation of MSCs **[Au:OK?]** into alveolar epithelial cells in the lesion. A recent study showed that MSCs can differentiate into respiratory epithelial cells (that is, type II alveolar cells).[24] To some extent, therefore, the differentiation of MSCs to endothelial cells might reconstruct the structure of the nephron, thereby improving renal function. Presently, however, no data can confirm or refute these hypotheses.

Conclusions

DAH is a rare complication of SLE that can occur at any time during the course of the disease. First-line treatment with high-dose corticosteroids has limited efficacy. In this Case Study, we show that MSCT was well tolerated by a patient with SLE-associated DAH, and resulted in disease remission. Considering that the disease continued to deteriorate despite two cycles of treatment with high-dose corticosteroids, that the patient showed rapid and marked improvement after MSCT, particularly after the second course of MSCT following a relapse of DAH, and that the doses of prednisolone and immunosuppressive agents used were unchanged throughout the intervention, we believe that it was the MSCT rather than the corticosteroid therapy that exerted a potent therapeutic effect. MSCT could, therefore, represent a promising future therapeutic strategy for treating DAH in patients with SLE.

1. Santos-Ocampo, A. S., Mandell, B. F. & Fessler, B. J. Alveolar hemorrhage in systemic lupus erythematosus: presentation and management. *Chest* **118**, 1083–1090 (2000).
2. Zamora, M. R. *et al.* Diffuse alveolar hemorrhage and systemic lupus erythematosus. *Medicine* **76**, 192–202 (1997).
3. Guleria, R. & Pangtey, G. Lung in SLE. *Indian Journal of Rheumatology* **4**, 131–132 (2007).
4. Green, R. J. *et al.* Pulmonary capillaritis and alveolar hemorrhage. *Chest* **110**, 1305–1316 (1996).
5. Todd, D. J. & Costenbader, K. H. Dyspnoea in a young woman with active systemic lupus erythematosus. *Lupus* **18**, 777–784 (2009).
6. Cañas, C. *et al.* Diffuse alveolar hemorrhage in Colombian patients with systemic lupus erythematosus. *Clin. Rheumatol.* **26**, 1947–1949 (2007).
7. Chang, M. Y. *et al.* Diffuse alveolar hemorrhage in systemic lupus erythematosus: a single center retrospective study in Taiwan. *Ren. Fail.* **24**, 791–802 (2002).
8. Wu, C. Y. *et al.* Severe pulmonary hemorrhage as the initial presentation in systemic lupus erythematosus with active nephritis. *Lupus* **10**, 879–882 (2001).
9. Santos, B. H. *et al.* Pulmonary hemorrhage as a manifestation of systemic lupus erythematosus. *Rev. Hosp. Clin. Fac. Med. S. Paulo* **59**, 47–50 (2004).
10. Zhang, B. *et al.* Mesenchymal stem cells induce mature dendritic cells into a novel jagged-2-dependent regulatory dendritic cell population. *Blood* **113**, 46–57 (2009).
11. Nauta, A. J. & Fibbe, W. E. Immunomodulatory properties of mesenchymal stromal cells. *Blood* **15**, 3499–3506 (2007).
12. Prigione, I. *et al.* Reciprocal interactions between human mesenchymal stem cells and γδ T cells or invariant natural killer T cells. *Stem Cells* **27**, 693–702 (2009).
13. Sotiropoulou, P. A. *et al.* Interactions between human mesenchymal stem cells and natural killer cells. *Stem Cells* **24**, 74–85 (2006).
14. Le Blanc, K. *et al.* Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet* **371**, 1579–1586 (2008).
15. Sun, L. Y. *et al.* Abnormality of bone marrow-derived stem cell in patients with systemic lupus erythematosus. *Lupus* **16**, 121–128 (2007).
16. Zhou, K. X. *et al.* Transplantation of human bone marrow mesenchymal stem cell ameliorates the autoimmune pathogenesis in MRL/lpr mice. *Cell. Mol. Immunol.* **5**, 417–424 (2008).
17. Sun, L. Y. *et al.* Mesenchymal stem cell transplantation reverses multi-organ dysfunction in systemic lupus erythematosus mice and humans. *Stem Cells* **27**, 1421–1432 (2009).
18. Zhao, F. *et al.* Therapeutic effects of bone marrow-derived mesenchymal stem cells engraftment on bleomycin-induced lung injury in rats. *Transplant. Proc.* **40**, 1700–1705 (2008).
19. Gupta, N. *et al.* Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. *J. Immunol.* **179**, 1855–1863 (2007).
20. Weiss, M. L. & Troyer, D. L. Stem cells in the umbilical cord. *Stem Cell Rev.* **2**, 155–162 (2006).
21. Lu, L. L. *et al.* Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoiesis-supportive function and other potentials. *Hematologica* **91**, 1017–1026 (2006).
22. Valencia, X. *et al.* Deficient $CD4^+CD25^{high}$ T regulatory cell function in patients with active systemic lupus erythematosus. *J. Immunol.* **178**, 2579–2588 (2007).
23. Nalbandian, A., Crispín, J. C. & Tsokos, G. C. Interleukin-17 and systemic lupus erythematosus: current concepts. *Clin. Exp. Immunol.* **157**, 209–215 (2009).
24. Berger, M. J. *et al.* Differentiation of umbilical cord blood-derived multilineage progenitor cells into respiratory epithelial cells. *Cytotherapy* **8**, 480–487 (2006).

Acknowledgments

The authors are grateful to the patient's family for granting permission to write this case report. They thank Dr Xiang Hu and Shengqin Ye (Jiangsu Stem Cell Bank, Jiangsu, China) for providing the MSCs. They thank Dr Gary S. Gilkeson (Department of Rheumatology, Medical University of South Carolina, Charleston, SC, USA) for his critical review of the manuscript. This work was supported by grants from the National Natural Science Foundation of China (No. 30772014 and No. 30972736), Jiangsu Province 135 Talent Foundation (RC2007002), Jiangsu Province Natural Science Foundation (No. 09KJB320010), Jiangsu Province Science-Technology Achievement Transforming Foundation (BA2009124), Jiangsu Province "Six Summit Talent" Foundation and Nanjing Health Bureau Young Scientists Launching Project (QYK09174).